A structured review of baculovirus infection process: integration of mathematical models and biomolecular information on cell–virus interaction

Abha Saxena, Prasanna Kumar Byram, Suraj Kumar Singh, Jayanta Chakraborty, David Murhammer and Lopamudra Giri

Abstract

The baculovirus expression vector system (BEVS) is an emerging tool for the production of recombinant proteins, vaccines and bio-pesticides. However, a system-level understanding of the complex infection process is important in realizing large-scale production at a lower cost. The entire baculovirus infection process is summarized as a combination of various modules and the existing mathematical models are discussed in light of these modules. This covers a systematic review of the present understanding of virus internalization, viral DNA replication, protein expression, budded virus (BV) and occlusion-derived virus (ODV) formation, few polyhedral (FP) and defective interfering particle (DIP) mutant formation, cell cycle modification and apoptosis during the viral infection process. The corresponding theoretical models are also included. Current knowledge regarding the molecular biology of the baculovirus/insect cell system is integrated with population balance and mass action kinetics models. Furthermore, the key steps for simulating cell and virus densities and their underlying features are discussed. This review may facilitate the further development and refinement of mathematical models, thereby providing the basis for enhanced control and optimization of bioreactor operation.

INTRODUCTION

Baculoviruses are a diverse group of viruses that infect insects and other invertebrates. These viruses contain a single molecule of circular supercoiled double-stranded DNA that varies in size from 80 to 180 kb and encodes for 100–200 proteins [1]. The genome is packaged into rod-shaped virions that are typically 250–300 nm in length and 30–60 nm in diameter. The virions are present as two phenotypes: (a) occlusion-derived virus (ODV) and (b) budded virus (BV) [2].

Baculoviruses, primarily Autographa californica multiple nucleopolyhedrovirus (AcMNPV), are widely used in conjunction with insect cell cultures to produce recombinant proteins [3]. This baculovirus/insect cell system is commonly referred to as the baculovirus expression vector system (BEVS). Since its development in the 1980s [4, 5], the BEVS has been widely used in laboratories to study protein structure and function. The BEVS has also been used to produce commercial products, particularly subunit vaccines for human and veterinary use. Three human vaccines produced with the BEVS have been approved by the FDA: (i) Cervarix, produced by GlaxoSmithKline, approved in 2009 as a cervical cancer vaccine; (ii) Provenge, produced by Dendreon, approved in 2010 for prostate cancer therapy; and (iii) Flubok, produced by Protein Sciences Corporation, approved in 2013 as an influenza vaccine. Furthermore, three products produced with the BEVS have been approved for veterinary use: Porcilis PCV (produced by Merck) and CircoFLEX (produced by Boehringer Ingelheim) have been approved as poxviruses as cocktails and Porcilis Pesti (produced by Merck) has been approved as a classical swine fever vaccine [6, 7].

In addition to their use in recombinant protein and vaccine production, baculoviruses can also be used as bio-pesticides.
for insect pest control. The primary advantages of using baculoviruses for insect control instead of chemical pesticides include (i) baculovirus specificity, i.e. they do not harm beneficial insects such as honey bees and are not hazardous to higher organisms, and (ii) environmental safety [8–10]. While the use of baculoviruses for insect control is limited, primarily due to the slow killing time and relatively high cost compared to chemical pesticides, they are used to control a variety of insect pests in many countries, including China and Brazil [9]. Certis USA (Columbia, MD, USA) is the primary commercial producer in the United States.

Although many papers have described various aspects of the baculovirus infection process, including many excellent review articles [11–16], there is a dearth of publications that describe the entire infection process, together with the mutations occurring during passing in reactors. Specifically, papers that discuss the available mathematical models, together with the biological details of the process, are particularly rare. The objective of this review is to summarize the entire baculovirus infection process and discuss the existing mathematical models in light of current knowledge. Such an effort is vital because experimental and theoretical studies must go hand in hand. Experiments are the only way to estimate parameters for the models and models are indispensable for the large-scale operation, control and optimization of bioreactors.

This review is organized into seven sequential modules: (1) internalization of the virus; (2) replication of viral DNA and early, late and very late protein expression; (3) formation of budded virus (BV); (4) formation of ODV; (5) formation of polyhedra (FP) and defective interfering particle (DIP) mutants during passing in bioreactor operation; (6) modification of cell cycle steps during viral infection; and (7) modification of the apoptosis process during viral infection. Mathematical models have been discussed alongside current experimental understanding of the field. In the next section the baculovirus phenotypes are briefly described and a brief overview of primary infection in insect larvae is provided, prior to discussion of the individual modules.

**BACULOVIRUS PHENOTYPES**

Baculoviruses are present as two phenotypes: (a) ODV is the virus form released from occlusion bodies (also known as polyhedra), i.e. a protein matrix in which one or multiple virus particles are embedded, and (b) BV is the virus form consisting of a single enveloped virus particle where the envelope is derived from the host cell membrane. The virus is present in the environment as occlusion bodies and hence ODVs initiate an infection in an insect host. On the other hand, BV helps in systemic infection, i.e. it transmits the virus from one cell to another within an insect. The interaction of these two phenotypes with cells is also different: ODVs are internalized by fusion with the plasma membrane (Fig. 1) [17], whereas BV is internalized by adsorptive endocytosis (Fig. 2) [17, 18].

These two forms of virus are also assembled at different times and in different cellular locations. The genes encoding BV proteins (e.g. vp39 and p6.9) are transcribed during the late phase [6–15 h post-infection (p.i.)], whereas the genes encoding polyhedral proteins (e.g. polyhedrin and p10) are primarily transcribed during the very late phase (>15 h p.i.) of infection [19]. Thus, BVs mature early in the infection and derive their envelope from modified cell membranes and ODVs mature late in the infection and acquire their envelope within the nucleus.

**NOMENCLATURE**

- $a$: surface area for growth per unit reactor volume [20]
- $A_j$: dimensionless ‘free’ area or unoccupied surface area [20]
- $b$: rate of virus burst from cells [21]
- $C$: cell density [22]
- $c$: number of cells [23]
- $c_d$: cell division rate [21]
- $e^{-\psi_{d1}}$: probability of a cell not becoming infected by DIPs [23]
- $e^{-\psi_{d2}}$: probability of a cell not becoming infected by helper virus [23]
- $k$ (increase in cell death rate corresponding to 10 intracellular vDNA copies) [19]
- $k_{\ldots\ldots}$: crosslinking reverse rate constant [22]
- $k_a$: attachment rate constant [19]
- $k_{d}$: cell death rate [19]
- $k_{d1}$: intrinsic cell death rate [19]
- $k_{d2}$: death rate of infected cells (h$^{-1}$) [24]
- $k_{d3}$: increase in cell death rate due to infection [19]
- $k_{dRNA,j}$: first-order mRNA $j$ degradation rate [19]
- $k_{er}$: constitutive endocytosis rate constant of receptor [22]
- $k_{ev}$: endocytosis rate constant of virus [22]
- $k_f$: 3D forward rate constant for a single VAP [22]
- $k_{fus}$: fusion rate constant [22]
- $k_t$: transport rate constant of virus to nucleus [22]
- $k_i$: infection rate constant [20]
- $k_j$: 3D dissociation rate constant for a single VAP [22]
- $k_{RDNA}$: first-order vDNA replication constant [19]
- $k_{rec}$: recycle rate constant of intracellular free receptors to cell surface [22]
- $k_r$: receptor synthesis rate constant [22]
- $k_{SRNA,j}$: first order transcription rate [19]
A schematic of the primary infection process in insect larvae (e.g. caterpillars) is shown in Fig. 1. The insect gastrointestinal tract at the initial site of infection can be divided into the foregut, midgut and hindgut (Fig. 1a). The foregut is involved in the uptake and storage of ingested material. The midgut is mainly the site for digestion and is lined with the peritrophic matrix (PM), which is composed of chitin, mucopolysaccharides and protein, whereas the hindgut is involved in the uptake of digestive material [26]. The polyhedra ingested by the insects are digested through enzymes and ions and the process is regulated by pH. Although the pH is 7 at entry and exit, it varies in the midgut from 10 to 12 [27]. The alkaline conditions in the midgut dissolve the polyhedrin and release ODVs (Fig. 1b). Further degradation of the polyhedrin takes place through proteinases, thereby releasing occluded viruses.

A set of proteins known as per os infectivity factors, e.g. ODV envelope proteins, are unique to ODVs and are not found in BVs. The major role of these proteins [Ac138 (p74-pif), Ac22 (pif-2), Ac119 (pif-1), Ac115 (pif-3), Ac96 (pif-4), Ac148 (ODV-E56, pif-5), Ac68 (pif-6)] (Table 1) is to mediate...
the specific binding of ODV to midgut cells [28–30]. When ODVs bind to proteinase-sensitive receptors, the ODV envelope fuses with the epithelial cell membrane and releases the nucleocapsids into the cytoplasm of the cell [17] (Fig. 1).

Lepidopteran nucleopolyhedrosis viruses (NPVs) are separated into two major groups based on their gene content [31]. The BV viral envelope contains two major viral proteins, namely F protein (Ac23) and GP64 (Ac128) (Table 2a) for group I NPVs [32, 33]. The membrane of group II NPVs that lack gp64 are modified by homologues of the F protein [34]. Virus budding and secondary infections require modification of the host cell membrane by GP64 [35, 36]. As discussed previously, the infection process is divided into several modules, and first in this sequence is virus internalization.

**VIRUS INTERNALIZATION**

The internalization of BVs involves several steps [22], as shown in Fig. 2. These involve binding to the extra cellular membrane (ECM), transport within cells consisting of endocytosis, endosomal transportation and nuclear pore formation. The key protein involved in initiating the infection process, as well as exit from cells, is GP64 (Table 2a). GP64 mainly regulates the entry of AcMNPV via clathrin-mediated endocytosis [37]. In this process, BVs bind to receptors that are present on the surface of clathrin-coated structures, where clathrin is present in the pits on the surface of the plasma membrane (Fig. 2a). When a BV binds to these receptors containing internalization signals, the pit is internalized into the cell cytoplasm (Fig. 2b). This internalized vesicle is known as an endocytic vesicle or an endosome, and this endocytic vesicle is acidified after internalization, which causes conformation changes in the viral fusion protein (GP64) (Fig. 2c). This further results in the fusion of the viral envelope with the endosomal membrane and the subsequent release of nucleocapsids into the cell cytoplasm (Fig. 2d). After the nucleocapsids enter into the cytoplasm, they are transported to the nuclear membrane by the process of actin polymerization, which involves viral P78/83 capsid protein and the host Arp2/3 complex (Table 2a) (Fig. 2e) [38, 39]. Several pieces of evidence suggest that the nucleocapsids then enter the nucleus through the nuclear pore complex [40].

Although a large amount information is available regarding the proteins involved in the internalization process, study of the protein dynamics through modelling and simulation is somewhat lacking. A mathematical model that describes the
virus internalization during early infection was proposed by Dee and Shuler [22]. The internalization process was modelled through the consideration of several steps: attachment, internalization, endosomal fusion, lysosomal routing, nuclear accumulation and multivalent bond formation of baculovirus in Sf21 insect cells. The events are similar to those described in Fig. 2 and the corresponding equations are summarized here.

The outermost species in the process of internalization is the extracellular virus. The rate of change of extracellular virus concentration may be written as:

\[
\frac{dV_{ex}}{dt} = - (\alpha k_F C) V_{ex} R_{sf} + k_r V_1
\]  

(1)

The first term in equation 1 is the usual second order mass action kinetics involving total extracellular virus concentration and total cell concentration. Viruses attached with a viral attachment protein (VAP) may be dislodged, which gives rise to the second term. It may be noted that if the virus develops more than one bond, it cannot be dislodged.

The next species to consider is the surface-attached virus \((V_s)\). As expected, it will contain the attachment term and a loss term. The attachment term corresponds to the process through which the virions from extracellular environment attach to the cell surface by appropriate binding with the receptors. The loss term corresponds to the encapsulation of the virions in the vesicles (Fig. 2). This loss term has been taken to be proportional to the surface virus \((V_s)\) present:

\[
\frac{dV_s}{dt} = (\alpha k_F C) V_{ex} R_{sf} - k_V V_1 - k_{sp} V_s
\]  

(2)

Table 1. per os infectivity factors

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Protein</th>
<th>Function</th>
<th>Effect of deletion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ac138 (p74, pif-0)</td>
<td>Oral infection in insects, mediates binding of ODV to midgut cells</td>
<td>Mutants were infectious (in vitro) but were not infectious (in vivo)</td>
<td>[94]</td>
</tr>
<tr>
<td>2.</td>
<td>Ac119 (pif-1)</td>
<td>Binding of ODV virus to midgut cells</td>
<td>Mutants were infectious (in vitro) but were not infectious (in vivo)</td>
<td>[94]</td>
</tr>
<tr>
<td>3.</td>
<td>Ac22 (pif-2)</td>
<td>Binding of ODV virus to midgut cells</td>
<td>Mutants were infectious (in vitro) but were not infectious (in vivo)</td>
<td>[94]</td>
</tr>
<tr>
<td>4.</td>
<td>Ac115 (pif-3)</td>
<td>ODV envelope-associated protein</td>
<td>Mutants were infectious (in vitro) but were not infectious (in vivo)</td>
<td>[95]</td>
</tr>
<tr>
<td>5.</td>
<td>Ac96 (pif-4)</td>
<td>ODV envelope-associated protein</td>
<td>Virus could replicate (in vitro), but not (in vivo)</td>
<td>[96]</td>
</tr>
<tr>
<td>6.</td>
<td>Ac148 (ODV-E56, pif-5)</td>
<td>Localizes in the envelopes of occluded virions</td>
<td>Virus could replicate (in vitro), but not (in vivo)</td>
<td>[97, 98]</td>
</tr>
<tr>
<td>7.</td>
<td>Ac68 (pif-6)</td>
<td>Involved in polyhedron morphogenesis</td>
<td>Longer lethal time (in vivo) produced abnormal polyhedra and lacked virions</td>
<td>[65, 99]</td>
</tr>
</tbody>
</table>

Table 2. Viral proteins involved in infection

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Gene</th>
<th>Function</th>
<th>Effect of deletion</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Virus internalization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Ac23 (F protein)</td>
<td>BV envelope fusion protein</td>
<td>More single enveloped nucleocapsids were produced</td>
<td>[56]</td>
</tr>
<tr>
<td>2.</td>
<td>Ac128 (GP64)</td>
<td>Entry of BV into cells and exit from cells; initiating infection to other cells</td>
<td>Viruses replicated in a single cell, could not bud out and infect surrounding cells</td>
<td>[35, 36]</td>
</tr>
<tr>
<td>3.</td>
<td>Ac9 (P78/83)</td>
<td>Involved in nuclear actin assembly; responsible for movement of virions through cytoplasm; association of ODV with envelopes</td>
<td>Defect in actin polymerization with reduced actin tails; irregular paths of movement with changed direction</td>
<td>[100]</td>
</tr>
<tr>
<td>4.</td>
<td>Ac34 (Arp2/3)</td>
<td>Localizes to both the nuclei and cytoplasm of infected cell; induces actin polymerization in nucleus to help in virus replication</td>
<td>Delay in late gene expression</td>
<td>[101, 102]</td>
</tr>
<tr>
<td>(b) Budded virus formation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Ac89 (vp39)</td>
<td>Major capsid protein; transport of nucleocapsids destined to become BVs to the cell membrane</td>
<td>Resulted in no BV production</td>
<td>[103]</td>
</tr>
<tr>
<td>6.</td>
<td>Ac100 (p6.9)</td>
<td>Arginine-serine/threonine-rich DNA-binding protein</td>
<td>Nucleocapsids were not produced</td>
<td>[104]</td>
</tr>
<tr>
<td>(c) Occlusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Ac8 (polyhedrin)</td>
<td>Occlusion body protein; stabilize baculovirus virions in the environment</td>
<td>Nonessential (in vitro); polyhedrin locus used as the site for production of recombinant proteins</td>
<td>[4, 5]</td>
</tr>
<tr>
<td>8.</td>
<td>Ac137 (P10)</td>
<td>Required for the proper formation of the polyhedron envelope</td>
<td>Fragile and rough surface polyhedra</td>
<td>[62, 105, 106]</td>
</tr>
<tr>
<td>9.</td>
<td>Ac131 (Pp34) polyhedron envelope (PE)</td>
<td>Seals the surface of polyhedra and enhances their stability</td>
<td>Fragile and rough surface polyhedra</td>
<td>[105]</td>
</tr>
</tbody>
</table>
Next is the endosomal virus (\(V_{\text{endosome}}\), shown in Fig. 2), whose balance can be written as:

\[
\frac{dV_{\text{endosome}}}{dt} = k_v V_i - k_{\text{fus}} V_{\text{endosome}} - k_{\text{trans}} V_{\text{endosome}} \tag{3}
\]

It has two loss terms (equation 3): one corresponds to the fusion of the virus with the endosomal membrane and the other is for the transport of the virus to the lysosome. The fusion process releases viruses into the cytosol. Hence, the accumulation of viruses in the cytosol (\(V_{\text{cytosol}}\)) can be written as:

\[
\frac{dV_{\text{cytosol}}}{dt} = k_{\text{fus}} V_{\text{endosome}} - k_a V_{\text{cytosol}} \tag{4}
\]

Clearly, the loss term in equation 4 is the flux of viruses into the nucleus (\(V_{\text{nucleus}}\)), which has been written as a first order process:

\[
\frac{dV_{\text{nucleus}}}{dt} = k_a V_{\text{cytosol}} \tag{5}
\]

One of the key proteins in the entire internalization process is the receptor protein denoted by \(R_{fib}\) in the above equations. Dee and Shuler also wrote a balance for this by accounting for the binding of various species at the surface. The concentration of various surface species can be written as:

\[
\frac{dV_i}{dt} = (\alpha k_j C) V_a R_{fib} - k_v V_i - (j - 1) k_v V_i R_{fib} + 2k_{-x} V_j - k_v V_i \tag{6}
\]

\[
\frac{dV_j}{dt} = (j - i + 1) k_v V_{j-1} R_{fib} - (j - i) k_v V_i R_{fib} - i k_{-x} V_i + (i + 1) k_{-x} V_{j+1} - k_v V_i \tag{7}
\]

\[
\frac{dV_j}{dt} = k_a V_j R_{fib} - j k_{-x} V_j - k_v V_j \tag{8}
\]

where \(V_i\) is the number of viruses with \(i\) bound receptors per virus and \(j\) is the maximum number of receptors that can be bound per virus. Now, once the consumption of surface receptors is linked with the extracellular virus concentration, the receptor protein (\(S_{fib}\)) balance can be written as:

\[
\frac{dS_{fib}}{dt} = - (\alpha k_j C) R_{fib} V_a + k_v V_i - k_v R_{fib} \sum_{j=1}^{i} (j-i) V_i + k_{-x} \sum_{i=2}^{j} i V_i - k_v R_{fib} + k_{-x} R_{fib} + k_v R_{fib} \tag{9}
\]

It is clear that the first four terms in equation 9 correspond to loss of receptors by coupling with viruses. The fifth term is due to additional receptors that are lost from the cell surface while forming the endosome. The last two terms are due to the release of receptors from the endosome and the synthesis of fresh receptors, respectively. With this the model forms a closed set.

It is interesting to note that an analytical solution was obtained for the model equations and an excellent match with experiments was found for this model, although 14 fit parameters were used. The fit parameters seem to be within the expected values. However, this model does not consider the subsequent steps, such as DNA replication, mRNA expression and protein synthesis during the infection process.

**REPLICATION**

The next major event after internalization comprises the viral DNA replication and transcriptional processes of early, late and very late proteins. Fig. 3 shows the specific binding steps involved in the DNA replication and Fig. 4 shows the different phases of transcription. The first step is the uncoating of the DNA after the entry of the nucleocapsids into the nucleus. Later, viral DNA replication and transcription take place, resulting in the production of nucleocapsids that undergo packaging to form virogenic stroma, a site of viral genome replication and nucleocapsid assembly [41].

**Early events in transcription**

The replication process is initiated by employing enhancers and transcriptional activators that exploit the host cell transcriptional machinery. The baculovirus genome contains homologous regions (hrs) that act as transcriptional enhancers and originators of DNA replication (Fig. 3a) [42-46]. The transcriptional activator IE1 (Ac147) (Table 3) is an immediate early gene that is transcribed early in infection and binds to homologous regions. It regulates transient DNA replication [47, 48] and continues to be transcribed through the late phase [49]. During the early infection process, transcription is carried out by the host RNA polymerase to produce the components necessary for initiating viral DNA replication (Fig. 4a).

**DNA replication**

Two mechanisms are known to be used in baculovirus genome replication: (a) rolling-circle replication [43] and (b) recombination-dependent replication [50]. The specific binding reactions involved in DNA replication include helicase binding to DNA strands, binding of protein [single-stranded DNA-binding protein (SSB), Table 3] to single-stranded DNA, and binding of DNA primase and DNA polymerase (Fig. 3b, c). The six essential genes involved in baculovirus DNA synthesis include *ie1, helicase (p143), dna-pol* and late expression factors *lef-1, lef-2* and *lef-3* (Table 3) [47, 48].

**Late transcription and genome processing**

Baculovirus RNA polymerase consists of four subunits and transcribes late and very late genes. The late genes, e.g. Ac89 (vp39) and Ac100 (p6.9), are involved in late infection (Fig. 4b), whereas very late factor 1 (VLF-1) (Ac77) influences the hyper-expression of very late genes such as polyhedrin and p10 (Fig. 4c) [51]. These proteins are involved in polyhedra formation (Table 3). Additionally, it has been
suggested that VLF-1 is required for the regular assembly of capsids and the DNA packaging process [52, 53].

As our experimental knowledge concerning DNA replication and transcription progresses and researchers become increasingly aware of the various steps involved in the process, the construction of a mathematical model for replication and transcription has become feasible. In this context, Roldao et al. presented a model in which the details of the replication and very late protein expression were included, along with the infection. The key quantities for this model are: the number of infected cells, the number of uninfected cells on a per cell basis. One important point here is the fact that the amount of DNA in the nucleus is dependent on the extracellular viral concentration in the past, because it requires time for its transport to the nucleus. This delay is accounted for by writing \( V_j(t - \tau_{\text{traf}}) \). The second term in equation 12 represents the viral DNA replication kinetics to first order with an explicit time-dependent function.

The key issue addressed in this paper is the dynamics of viral DNA in the nucleus (DNA/cell) of the cells (\( DNA_n^{\text{rep}} \)). Clearly, this is a vital quantity for the quantification of replication. The equation for this quantity is given by:

\[
\frac{dT_{\text{DNA}}}{dt} = \eta_{\text{traf}} k_{\text{DNA}} [V_j(t - \tau_{\text{traf}})(N_i(z) + N_u) \left( \frac{N}{N} \right) + k_{\text{DNA}}^{\text{RNA}}(t, \delta_{\text{DNA,low}}, \delta_{\text{DNA,high}})]
\]

As discussed above, \( V_j \) in equation 12 represents the extracellular virus concentration, where \( j \) is one of the three viral proteins attached to the virion. The term in the square brackets in equation 12 is the total number of viruses internalized for \( N_i \) number of infected cells. Hence, the entire first term (equation 12) on the right corresponds to the transport of viral DNA from extracellular virus to the cells on a per cell basis. One important point here is the fact that the amount of DNA in the nucleus is dependent on the extracellular viral concentration in the past, because it requires time for its transport to the nucleus. This delay is accounted for by writing \( V_j(t - \tau_{\text{traf}}) \).

The mRNA synthesis (\( (RNA) \)) for the transcription of genes from the DNA template follows similar kinetics:

\[
\frac{dRNA}{dt} = k_{\text{DNA}}^{\text{RNA}}[f_{\text{DNA}}(t, \delta_{\text{DNA,low}}, \delta_{\text{DNA,high}}) - k_{\text{DNA}}^{\text{RNA}}(RNA)_{\text{rep}}(t, \delta_{\text{DNA,low}}, \delta_{\text{DNA,high}})]
\]

Intracellular viral protein synthesis (\( VP_{\text{rep}} \)) is given by Michaelis–Menten kinetics on intracellular RNA:

\[
\frac{dVP_{\text{rep}}}{dt} = K_{\text{VP}} + RNA_{\text{rep}} \times f_{\text{VP}}(\delta_{\text{VP,low}}, \delta_{\text{VP,high}})
\]

The key feature of this model is the temporal control of very late protein expression through the time-varying function incorporated in the formation of mRNA as well as protein.

There is another similarly structured model for recombinant protein production in batch and fed-batch cultures of baculovirus-infected insect cells. The model considers the key variables, including enveloped virus, adsorbed virus, nucleocapsid, viral nucleic acid, enzyme, mRNA, viral protein capsid, product protein and P10 protein inside the cytoplasm [54]. The simulation results for medium components, including recombinant protein, substrate and 20 amino acids, were fitted with the experimental data, implying that the model was able to capture the real metabolic reactions during the infection process. Additionally, the model...
provides the key features of viral infection in batch and fed-batch culture and predicts the intracellular concentrations of DNA, mRNA and enzymes.

Another model for baculovirus protein expression was discussed by Sanderson [55]. This models the concentrations of substrates, key metabolites (including fatty and amino acids, sugars, and intermediates of the glycolysis and tricarboxylic acid cycle) and mRNA expression. The mRNA expression was modelled as a function of substrate concentration. The equations were formulated for three regions: the growth medium, the cytoplasm and the mitochondria.

Table 3. Viral proteins involved in replication and transcription

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Gene</th>
<th>Function</th>
<th>Effect of deletion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ac147 (IE1)</td>
<td>Transcriptional activator that binds to hrs; regulates transient DNA replication</td>
<td>Mutant was not viable, so it is an essential gene</td>
<td>[47–49, 107]</td>
</tr>
<tr>
<td>2.</td>
<td>Ac95 helicase (p143)</td>
<td>Unwinding the DNA by disrupting the hydrogen bonds between bases in the double helix</td>
<td>No DNA synthesis</td>
<td>[108]</td>
</tr>
<tr>
<td>3.</td>
<td>Ac65 (dnapol)</td>
<td>DNA polymerase that extends DNA from the 3’ OH of a primer in the 5’ to 3’ direction</td>
<td>No viral DNA replication was observed</td>
<td>[110]</td>
</tr>
<tr>
<td>4.</td>
<td>Ac14 (lef-1)</td>
<td>DNA primase, helps in generation of primer; initiates DNA replication process; also interacts with LEF-2</td>
<td>An essential gene as deletion/insertion mutant could not be isolated</td>
<td>[103, 111–113]</td>
</tr>
<tr>
<td>5.</td>
<td>Ac6 (lef-2)</td>
<td>Primase-associated factor; involved in both replication and very late transcription</td>
<td>Unable to produce infectious virus</td>
<td>[114]</td>
</tr>
<tr>
<td>6.</td>
<td>Ac67 (lef-3)</td>
<td>Single-stranded DNA-binding protein (SSB); also facilitates transport of helicase into the nucleus</td>
<td>Limited amounts of DNA replication and late gene expression</td>
<td>[29, 115–117]</td>
</tr>
<tr>
<td>7.</td>
<td>Ac77 (VLF-1)</td>
<td>Influences the hyper-expression of very late genes; present in both BV and ODV; required for the production of nucleocapsids</td>
<td>Capsids produced lacked DNA; nucleocapsids were unable to release from virogenic stroma (involved in DNA maturation)</td>
<td>[51, 90, 118]</td>
</tr>
</tbody>
</table>
It can be seen that although these models provide quantitative information about the viral DNA loading and the quantity of viral proteins, they do not quantify the process of BV formation. Specifically, these models do not consider the mechanisms for the formation of the two morphologies, i.e. BV and ODV.

**FORMATION OF BVS**

A schematic diagram showing the formation and release of BV is shown in Fig. 5. When the transcriptional cascade is initiated in the nucleus (Fig. 5a), it results in the production of nucleocapsids that assemble to form virogenic stroma [41] (Fig. 5b). Viral envelope protein GP64 and F protein are synthesized and translated in association with the endoplasmic reticulum (Table 2a) [56]. These proteins are glycosylated in the Golgi apparatus and are transported to the cytoplasmic membrane via transport vesicles (Fig. 5c, d). Genes encoding BV proteins (vp39, p6.9) are transcribed cytoplasmic membrane via transport vesicles (Fig. 5c, d). During the late phase (Table 2b). Further, it has been suggested that while the virus buds out from nucleus, the nucleocapsids obtain an envelope from the nuclear membrane (Fig. 5e), which is lost during transit through the cytoplasm (Fig. 5f) [40]. After reaching the modified cytoplasmic membrane, the virions obtain an envelope while budding through the membrane (Fig. 5g).

One of the early models for viral dynamics was proposed by Kumar and Shuler [20]. They modelled the transient behaviour of a split-flow airlift reactor that was used for the production of baculovirus. Because the cells were immobilized on the particle surface of a packed bed, the concentrations varied with the bed height, z. Although the budding process was not explicitly present, they considered the dynamics of extracellular virions and non-occluded virus.

The concentrations of infected \( N_{\text{inf}}(z, t) \) and uninfected cells \( N_{\text{uninf}}(z, t) \), extracellular virions \( V_{\text{syn}}(z, t) \) and non-occluded viruses \( V_{\text{syn}, T}(z, t) \) are the four key variables in the model. Since the time that has elapsed after infection is an important attribute for a cell, the concentration of cells that suffered infection for a period \( \tau \) was used as an additional variable. This is denoted by \( \{ dN_{\text{inf}}(z, \tau) \} \). The dynamic equations for cells and viruses are written as typical mass action or saturation kinetics. The dynamics of the infected cell is given by:

\[
\frac{dN_{\text{inf}}(z, t)}{dt} = k_i N_{\text{uninf}}(z, t) \times \frac{V_{\text{syn}}(z, t)}{(N_{\text{uninf}}(z, 0) aK_v + V_{\text{syn}}(z, t))}
\]  

This particular form ensures the shifting order. When the virus population is very low, the rate is solely dependent on extracellular virus concentration, but if the virus population is very high, it depends solely on cell concentration. The dynamics of the uninfected cell can be written as the sum of the growth and infection terms. A slightly modified form of Monod kinetics is used for the growth term and is omitted here.

The total rate of synthesis of non-occluded virus inside an infected cell is given by:

\[
V_{\text{syn}, T}(z, t) = \sum_{i=0}^{t} A_i(z, t) V_{\text{syn}}(t - t_i, N_i) dN_{\text{inf}}(z, t_i) dt
\]  

Here \( t_i \) is the period for which the cell remained infected. The function \( V_{\text{syn}}(t, N) \) is the rate of synthesis of non-occluded virus for a cell that is infected for a period \( t \) under the overall culture cell density \( N \). This function is known from independent experiments.

The net rate of release of virions in the extracellular environment is given by the difference between the synthesis and infection rates:

\[
R(z, t) = V_{\text{syn}, T}(z, t) - \frac{\partial N_{\text{inf}}(z, t)}{\partial t}
\]  

This forms a closed set of equations that can be solved to obtain the infected and uninfected cell concentrations, as well as the viral loading. Once these quantities are known, the other required variables can be computed. For example, the rate of cell lysis \( N_{\text{lyse}}(z, t) \) is given by:

\[
N_{\text{lyse}}(z, t) = \sum_{i=0}^{t} dN_{\text{inf}}(z, t_i - t_i)
\]  

Finally, the cell kinetics has been coupled with nutrient fluxes through the transport equations. This model produces a satisfactory match with the experiments. Unavoidably, the model has a number of fit parameters, but the values of the parameters appear to be reasonable.

A number of population balance models also exist [25, 54] to explain the BV dynamics in the insect cells. Because the post-infection age of a cell is an important parameter, it can readily be considered using a population balance model:

\[
\frac{\partial n(t, \tau)}{\partial t} + \frac{\partial n(t, \tau)}{\partial \tau} = 0
\]  

where \( n(t, \tau) \) in equation 19 denotes the number of cells with a post-infection age \( \tau \) at time \( t \).

Sometimes additional attributes of a cell, such as multiplicity of infection (m.o.i.), are also included as an additional variable describing the cell population. For example, [57] included m.o.i. (NV) as an additional cell property and hence a balance equation for \( n(t, t, NV) / \partial t \) is needed. Such an inclusion may further complicate the model and a trade-off between accuracy and ease dictates the choice of model.

Population balance models usually yield better accuracy, since they allow accurate accounting of cell age, a key variable determining most of the events [25]. However, it is necessary to solve a partial differential equation to obtain the viral dynamics in these cases. The total infected cell concentration is readily obtained by integrating quantities such as
\[ n(t, \tau)/\tau \] and the other parts of the model are very similar to those discussed earlier. Although these models address the formation of non-occluded virus, they do not consider the formation of occluded virions and their packaging in polyhedra.

**OCCLUSION**

The baculovirus infection process involves ODV formation and packaging in polyhedra. The five steps (a, b, c, d and e) of the occlusion process are shown in Fig. 6.

**ODV formation**

The assembled nucleocapsids, which remain in the nucleus, undergo occlusion involving several very late proteins (p10, polyhedrin and PE). ODVs are formed in the nucleus by the envelopment of a single nucleocapsid or multiple nucleocapsids to form a virion. Virions remaining in nuclei are observed to obtain a membrane from microvesicles and can be further modified by incorporating virally encoded ODV-specific envelope proteins (Table 1) (Fig. 6b) [58]. Following initiation of the transcriptional cascade, mRNAs encoding the ODV envelope proteins are transcribed and exported to the cytoplasm for translation. These proteins are then transported from the cytoplasm to the nucleus, where the inter-nuclear membrane (INM) proliferates and buds into the nucleus to form microvesicles [58, 59]. It has been also suggested that the calcium flux within the infected cell nucleus helps to regulate the nuclear envelope breakdown. During infection, increased intranuclear \( \text{Ca}^{2+} \) may control the budding of the INM and the formation of these membranes, which serve as a source of ODV envelopes [58, 59].

**Polyhedra formation**

The function of the major viral proteins involved in the occlusion process is discussed in Table 2(c). During the final stage of baculovirus replication, the high levels of mRNA expression of very late genes leads to the production of p10 and polyhedrin (Table 2c). Polyhedra are occlusion body proteins that crystallize and accumulate in nuclei to
form a lattice for the assembly of ODVs [5]. A nuclear localization signal is required for the transport of polyhedra from the cytoplasm to the nucleus. High levels of P10 form tube-like structures that penetrate both the nucleus and the cytoplasm [60, 61]. As the occlusion bodies mature, P10 fibrils align with the polyhedra surfaces. Additionally, p10 is required for proper assembly of the polyhedron envelope (PE) on the surface of occlusion bodies. The inclusion of ODVs in polyhedra (Fig. 6d) helps to protect the virions from inactivation and they can survive for up to 20 years in the environment under favourable conditions [62–65]. However, one of the major obstacles for the economical production of baculovirus is the accumulation of mutants and the formation of empty polyhedra through passaging in the bioreactor [66].

**DIP AND FP MUTANTS**

The repeated passaging of baculovirus in cell culture in continuous bioreactors results in few polyhedra (FP) mutant accumulation [67, 68]. Fig. 7 shows the three major events involved in FP mutant formation. Typically, the FP phenotype of baculoviruses is characterized by few polyhedra/cells, few or no ODVs/polyhedra, increased BV production and altered intra-nuclear envelopment [69, 70]. Analysis of infected cell-specific proteins of FP mutants from AcMNPV suggests that the fp25k gene is mutated in the FP mutants (Fig. 7a) [71]. It has been indicated that the transposons/transposable elements of the host cell (mobile DNA segments that insert into a functional gene) are inserted into the fp25k gene, thereby causing mutations by disrupting the gene sequence [72].

DIPs [73] are mutant viruses that arise naturally from virus infection as a consequence of repeated passaging in cell culture. Figs 8 and 9 show a schematic diagram illustrating the formation and accumulation of DIPs, respectively [66]. Although the exact mechanism of DIP formation is not clear, homologous recombination is one possibility, which involves sequence overlap looping and subsequent excision of the non-hr ori region [74]. To date, nine hrs have been identified in the AcMNPV genome, designated as hr1, hr1a, hr2, hr2a, hr3, hr4a, hr4b, hr4c and hr5. They vary in size from 0.4 to 1.0 kb and have adenine-plus-thymine-rich sequences containing 2–8 and 30 bp imperfect palindromes with an EcoRI site as the palindromic core. DIPs have deleted genomes (Fig. 8b) and they are unable to replicate by themselves in host cells. DIPs will only replicate in cells that are co-infected with a helper virus that encodes for the genes required for replication that are missing in DIPs. When a cell is co-infected with DIPs and standard virus (i.e. helper virus), DIPs replicate faster than the standard virus, presumably due to their smaller genome size [21] (Fig. 9d). It has been shown that a high m.o.i. supports DIPs accumulation, since this increases the probability of a cell being co-infected by a DIP and a standard virus.

Various mechanisms for DIP formation have been proposed based on restriction enzyme analysis of DIP DNA (Fig. 8). The schemes proposed for DIP formation include recombination between hr sites (i.e. hr2, hr3, hr4 and hr5) [66]. A delay in DIP formation was observed when the transposon insertion sites were altered in the fp25k gene region (Table 4a). These results suggest that the insertion of transposons followed by the deletion of sequences between the inserted transposons may lead to DIP formation [66]. Since many of the FP mutant insertion sites are located within the deleted sequences of the DIPs, it is possible that FP mutants are the precursors of DIPs [75]. FP and DIP mutant accumulation during virus passaging in bioreactors leads to decreased polyhedra productivity and reduced virulence.

Although limited effort has been devoted to the mathematical modelling of DIP mutant formation specific to baculovirus production, Krikwood et al. discussed a model involving the infection of cells with STV, DIPs and both standard STV and DIPs [21]. This situation is depicted in Fig. 9. The model clearly shows how the proportion of DIPs changes with subsequent passaging. Like all other previous models, this model follows the first order kinetics for uninfected cells, infected cells and viruses, with an important distinction: the virus population is recognized as consisting of STVs and DIPs. Hence, cells can be infected by STVs, DIPs or both. As discussed above and shown in Fig. 9, only the first and third options can lead to an infection cycle, since the infection of cells solely with DIPs cannot produce progeny virus [21].

The kinetic equations for uninfected and DIP-infected cells are not given here. The main feature of the model comes while the population of cells infected by STV and both STV and DIP is being considered. In both cases, the age of the infected cells is considered and the entire population is divided into a large number of sub-groups with a narrow band of post-infection ages. The equation describing the dynamics of standard virus infected cell (CS) is:

\[
\frac{dC_{Si}}{dt} = \frac{C_{Si-1} - C_{Si}}{\tau} - C_{Si}p_{i} + a_{Si}VD_{j}z_{i}
\]  

The first term on the right-hand side of equation 20 represents the gain and loss of a class purely due to aging of the cells. The second term is a loss term corresponding to cell lysis and the last term is due to infection by a DIP. Once an STV-infected cell is infected by DIP, it becomes a cell that is infected by both (CBi), whose balance is written next:

\[
\frac{dC_{Bi}}{dt} = \frac{C_{Bi-1} - C_{Bi}}{\tau} - C_{Bi}p_{i}
\]  

The terms have similar origin to those above (equations 20 and 21), and only the last term is not relevant, since both infected cells remain as such until lysis. It can be noted that equations 20 and 21 are actually the discrete form of the population balance model discussed previously. The discrete form saves some of the effort of discretization, but does not offer any specific computational advantages.
The concentrations of STV increases because of cell lysis and decreases because of the infection of all types of cells present. These equations are also written as straightforward mass action kinetics. It may be noted that the release of STV (VS) and DIPs (VD) throughout the infected lifetime of the cell has not been considered in this model.

\[
\frac{dVS}{dt} = b \sum CS_i p_j - a.r VS \left( CU + \sum CS_i + CD + \sum CB_j \right) \tag{22}
\]

\[
\frac{dVD}{dt} = b \sum CB_j p_j - a.r VD \left( CU + \sum CS_i + CD + \sum CB_j \right) \tag{23}
\]

One important factor in the cell infection process during passage is the stochastic nature of the virion formation. To incorporate such stochasticity, Zwart et al. [23] took the number of virions per cell as a random variable distributed according to Poisson’s distribution,

\[
P(X) = \frac{e^{-\Psi} \Psi^X}{X!} \tag{24}
\]

where \(X\) is the number of virions per cell (helper virus or DIP; a random variable) and \(\Psi\) is the average number of virions per cell. The probability that a cell will not be infected is therefore obtained by setting \(X = 0\) and is given by \(e^{-\Psi_H}\) and \(e^{-\Psi_D}\) for helper virus and DIP, respectively. Hence, the probability that a cell will be infected will be \((1 - e^{-\Psi_H})\) and \((1 - e^{-\Psi_D})\) for these two classes. Cells (c) could be infected solely by helper virus (and not by DIP) or by both kinds, with the probability of each scenario being readily obtained as:

\[
\alpha = P(\text{infected by helper but not by DIP}) = e^{-\Psi_H} \left(1 - e^{-\Psi_D}\right) \tag{25}
\]

\[
\beta = P(\text{infected by helper and also by DIP}) = e^{-\Psi_H} \left(1 - e^{-\Psi_D}\right) \tag{26}
\]

The model gives the proliferation of helper virus and DIPS by a multiple of the existing viral load; \(\nu_\alpha\) and \(\nu_\beta\) for the two classes of infected cells, respectively. The corresponding terms are multiplied by the probability of DIP formation from helper virus-infected cells (\(\mu\)) and DIP- and helper virus-infected cells (\(1 - \phi\)). The proliferation upon passage is thus given by:
\[ n_H(t+1) = c(\alpha \nu_H (1-\mu) + \beta \nu_D \phi) \] (27)

\[ n_D(t+1) = c(\alpha \nu_D \mu + \beta \nu_D (1-\phi)) \] (28)

The mutation probability (\(\mu\)) has been modelled as a successful process (helper virus to helper virus) and a failed process (helper virus to DIP), and hence is given by a binomial distribution. This model is capable of predicting the oscillation of viral loading and the presence of chaotic behaviour in the system.

**MODIFICATION OF CELL CYCLE PHASES DURING BACULOVIRUS INFECTION**

The normal life cycle of the insect cell is characterized by a complex series of events ranging from cellular growth to replication and is known to be disrupted during the infection process. In general, the cell cycle involves various stages during which cell growth occurs in the gap or G phase (G1, G2 and G0), the synthetic or S phase and the mitotic or M phase [76]. In the G1 phase, the cells are prepared for DNA replication along with the production of synthetic enzymes, whereas the S phase involves DNA synthesis, during which chromosomes are duplicated. G2 prepares the normal cells for the cell division that occurs in the M phase. The transitions between the different stages of the cell cycle are governed by the phosphorylation state of the cyclin proteins that are phosphorylated by cyclin-dependent kinases (CDK) (Fig. 10a). It has been shown that baculovirus infection leads to the arrest of the cell cycle at the S or G2/M phase [77–79]. Additionally, it has been found that a viral protein EC27 (Ac144) (Table 4b) acts as a multifunctional cyclin (cyclin B and cyclin D) and plays a significant role in cell cycle arrest (Fig. 10b) (Table 4b). This protein is found in both viral progeny (i.e. ODVs and BVs) of AcMNPV [80]. Fig. 10 describes the difference between the cell cycle events of the uninfected and virus-infected cell cycles, respectively.

**G1/S phase**

In uninfected cells, Rb (retinoblastoma protein) remains bound with E2F (transcription factor) to regulate the G1/S checkpoint [81]. Cyclin D is known to phosphorylate Rb in association with Cdk6 and the resulting inactivated Rb releases E2F. The transcription factor E2F then regulates the gene transcription required for entry into the S phase (Fig. 10a, step G1) [82]. In contrast, during baculovirus infection viral protein EC27 (Ac144) (Table 4b) acts as a
cyclin D and forms Cdk6/EC27, which inactivates Rb. This allows cell cycle progression from the G1 to the S phase (Fig. 10b, step G1) [80].

**S phase**

Cell division cycle 6 (Cdc6) is known to be an essential regulator of DNA replication that is required for the assembly of pre-replicative (pre-RC) complexes at origins of replication (orc) during the G1 phase. In uninfected cells, the cyclin A/cdk2 complex causes cdc6 to dissociate from orc after replication initiation [83]. This ensures one genome replication and prevents further replication from the same origin (Fig. 10a, step S). During the infection, viral protein EC27 binds with host cdc6 to override the host checkpoint and allow repeated DNA replication (Fig. 10b, step S) [84].

**G2/M phase**

In uninfected cells, the association of cyclin B with Cdk1 is responsible for the breakdown of the nuclear envelope and the progression of a cell from the G2 to the M phase (Fig. 10a, step G2-M). However, during baculovirus infection, EC27 acts as cyclin B and forms the complex Cdk1/EC27 that does not allow the breakdown of the nuclear envelope, thereby arresting the cells in the G2/M phase (Fig. 10b, step G2-M). This arrest leads to ODV maturation, e.g. the formation of intranuclear microvesicles and viral envelopes (Fig. 6) [80].

**MODIFICATION OF APOPTOSIS DURING BACULOVIRUS INFECTION**

The baculovirus-mediated infection process is known to block apoptosis or programmed cell death and regulate the insect cell death pattern. In general, apoptosis allows insect cells to eliminate damaged or abnormal cells, thereby controlling infections. Fig. 11 shows the events occurring in the apoptosis of an uninfected cell and a virus-infected cell, respectively. For uninfected cells, the apoptosis involves the activation of two types of caspases, i.e. initiator caspases (ICs) and effector caspases (ECs). The ICs cleave the ECs to activate them. The activated ECs then cleave other signalling proteins, thereby resulting in apoptosis (Fig. 11c). It is known that baculovirus infection induces the expression of apoptotic inhibitors (e.g. p35 and p49) that prevent apoptosis by blocking caspase activity. The function of apoptotic genes is discussed in Table 4c.

**Anti-apoptotic gene**

During infection, viral protein p35 blocks the apoptotic pathway through binding with EC [85], whereas the p49 protein binds both ICs and ECs to block apoptosis (Fig. 11, step F-G) [86]. Furthermore, there are other apoptotic suppressors, such as Apsup, which blocks ICs in *Lymantria xylina* MNPV (Fig. 11, step H) [87].

Inhibitor of apoptosis (iap) is another anti-apoptotic protein family and the distribution of iap lineages (iap1, iap2, iap3, iap4 and iap5) varies among different baculovirus species.
In general, the iap proteins contain specific domains that bind to the caspases to block their function and thus prevent apoptosis. Specifically, iaps are characterized by baculovirus iap repeat (BIR) and really interesting new gene (RING) domains consisting of approximately 70 and 40 amino acids, respectively. The BIR domains of iaps are present with one to three copies, whereas the RING domains of iaps contain ubiquitin ligase activity to target caspases for ubiquitination and subsequent degradation [89]. However, to initiate apoptosis, iap antagonists (iap-A) counteract the anti-apoptotic activity of iap (Fig. 11, steps A, B).

Fig. 9. DIP accumulation during repeated passaging. (a, b) STV infects an uninfected cell and forms an STV-infected cell, which releases more STVs and fewer DIPs. (a, c) DIP infects an uninfected cell and forms a DIP-infected cell, which does not release anything. (a, d) DIP and STV infects an uninfected cell, forming a co-infected cell that releases more DIPs and fewer STVs.

Table 4. Viral proteins involved in mutation, cell cycle and apoptosis

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Protein</th>
<th>Function</th>
<th>Effect of deletion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) DIP and FP mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Ac61 (fp25k)</td>
<td>Polyhedrin biosynthesis and nuclear localization during the early occlusion phase (expression begins at ~18 h p.i.); targeting of nucleocapsids to cell nucleus; serves to ‘switch’ from BV to OV production</td>
<td>Fewer cells with polyhedra; polyhedra with few virions; abnormal occlusion process</td>
<td>[119, 120] [70]</td>
</tr>
<tr>
<td>2.</td>
<td>Ac144 (EC27)</td>
<td>Multifunctional cyclin; involved in cell cycle regulation during virus infection</td>
<td>Affects nucleocapsid formation but does not affect DNA synthesis</td>
<td>[80, 91]</td>
</tr>
<tr>
<td>(b) Modification of cell cycle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Modification of apoptosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Ac135 (p35)</td>
<td>Inactivates effector caspases</td>
<td>Mutants were viable; severely compromised in BV production</td>
<td>[121, 122]</td>
</tr>
<tr>
<td>4.</td>
<td>Ac142 (p49)</td>
<td>Inhibits both initiator and effector caspases</td>
<td>Failed to produce BV; produced polyhedra lacking virions; defective nucleocapsid formation</td>
<td>[123]</td>
</tr>
</tbody>
</table>
Comparison of uninfected and infected cell cycles. Uninfected cell cycle. (G1) Cyclin D/cdk4 complex phosphorylates Rb, which releases E2F, leading to the progression of the cell from the G1/S phase. (S) Cyclin A/cdk2 complex phosphorylates cdc6, which results in the release of E2F, leading to the progression of the cell from the G1/S phase. (G2) Cyclin B/cdk1 complex arrests the cell in the G2/M phase. Baculovirus-infected cell cycle. (G1) EC27/cdk4 complex phosphorylates Rb, which releases E2F, leading to the progression of the cell from the G1/S phase. (S) EC27 binds with the host’s cdc6, which results in repeated replication of the genome. (G2-M) EC27/cdk1 complex arrests the cell in the G2/M phase.

the activity of cellular iaps to facilitate apoptosis. In infected cells, baculovirus expresses a viral-iap, Op-iap3, which binds to iap-A antagonists instead of cellular iap (Fig. 11, step D–E). This in turn allows cellular iaps to remain bound with caspases to block apoptosis [90, 91].

Although there has only been limited mathematical modelling of the apoptotic process and the specific role of the proteins involved in baculovirus infection, unstructured models for cell death exist as part of infection models. Kirkwood et al. [21] described cell death in infected cells (CS) (infected solely with STV) and co-infected cells (CB) (infected with STV and DIPs as follows:

\[
dCS = a(\text{VS, CU}) - \frac{CS}{\tau} - \text{CS, p}(i) - a. \text{CS, VD, z}(i) \tag{29}
\]

\[
dCB = a. \sum \text{CS, VD, z}(i) + a. \text{VS, CD} - \frac{CB}{\tau} - \text{CB, p}(i) \tag{30}
\]

In the above equations, the third term in equation 29 and the fourth term in equation 30 denote the rate of loss of cells undergoing virus release because of cell lysis for infected cells (CS) and co-infected cells (CB), respectively. Generally, cell lysis is a common outcome of viral infection and consists of cell membrane disruption, which leads to cell death and the release of cytoplasmic compounds into the extracellular space.

The distribution of virus release times for the cells infected by STV (VS) was assumed to be governed by a factor \( p(i) \). \( p(i) \) can be defined as the risk of a cell undergoing virus-induced lysis as a function of \( i \), as shown below:

\[
if (i \leq i) p(i) = 0 \tag{31}
\]

\[
if (i > i) p(i) = \alpha \exp(\beta(i - i)) \tag{32}
\]

Here it is assumed that an infected cell passes through a minimum number of subclasses \( i \) before it is first exposed to the risk of lysis. Thereafter the rate of lysis increases exponentially in each successive subclass, where \( \alpha \) and \( \beta \) are the parameters controlling the distribution of release times.

Cell death can also be modelled through a time-dependent parameter [19]. In the case of uninfected cells \( (N_u) \), only the intrinsic death rate constant \( k_d \) is present, but in the case of infected cells \( (N_i) \) the term for both intrinsic death and death due to viral infection \( (k_{d1} + k_{d2}) \) are included.

\[
dN_u = -k_d N_u \left( \frac{1}{m. a. t.} \right) - k_{d1} N_u \tag{33}
\]

\[
dN_i = k_d N_u \left( \frac{1}{m. a. t.} \right) - k_{d1} N_i \tag{34}
\]

\[
k_d = k_{d1} if (t < \delta) \tag{35}
\]

\[
k_d = k_{d1} + k_{d2} if (t \geq \delta) \tag{36}
\]

In equations 33 and 34, the second term represents the first order death rate. For infected cells, during the first phase (equation 35) there is a slight decrease in infected cell concentration in relation to the intrinsic cell death rate. However, the second phase is mainly characterized by faster cell death due to viral infection (equation 35). \( \delta \) in equation 35 is the time at which the cell death rate increases. The cell death rate in the second phase, \( k_{d2} \), increases as the number of virions infecting the cells increases, as follows:

\[
k_{d2} = k' if (\text{DNA}_{\text{total}} \leq 10) \tag{37}
\]
$k_{d2} = k^*(DNA_{total})$ if $(DNA_{total}) > 10$ (38)

where, $k^*$ is the increase in the cell death rate, which is dependent on $DNA_{total}$ and $DNA_{total}$ is the total number of intracellular viral DNA copies.

A similar strategy was used for unstructured modelling of cell death by Power et al. [24]. The viable infected cells ($X_i$) obtained through the recombinant baculovirus infection were presented as follows:

\[
\frac{dX_i}{dt} = -q_0(T).X_i
\] (39)

\[
q_D(T) = K_{d2} if (0 < T < T_D)
\] (40)

\[
q_D(T) = +\infty, otherwise
\] (41)

In equation 40, the time-varying function, $q_D$ represents the death rate of the population at constant level, $K_{d2}$ (the death rate of the viable cells from the time of infection to $T_D$ (the time at which rapid death starts). Beyond this time (otherwise), the death rate of the infected cells becomes infinite (equation 41). The same group presented another model $i$ protein release was linked to the leakiness of the membrane and cell lysis [53]. Therefore, the following term was considered as the death of the infected cell:

\[
r_{pr}(\tau) = k_{pr}(\tau).p_i(\tau)
\] (42)

\[
k_{pr}(\tau) = 0, for \tau < \tau_U
\] (43)

\[
k_{pr}(\tau) = \alpha_{pr}, for \tau \geq \tau_U
\] (44)

where $r_{pr}$ is the protein release rate.
CONCLUSIONS AND OUTLOOK

There has been significant progress in deciphering new proteins that play roles in various key steps involved in the baculovirus infection process. A significant amount of information is available on the components of the biochemical pathway involved in virus internalization, replication, protein synthesis, BV and ODV formation, FP and DIP mutation formation, cell cycle disruption and modulation of apoptosis. Additionally, systems biologists have attempted to develop mathematical models for virus internalization, mRNA synthesis and protein synthesis. However, most of the models only describe one or two modules of the process involved in baculovirus infection. Very few models focus on the passaging effect that takes place in bioreactors and the mechanism of defective interfering particle formation [21, 23]. Moreover, limited effort has been put into developing mathematical models of FP mutation formation and the production of ODVs and polyhedra [92]. Although there is an integrated model for rotavirus-like VLP formation in the baculovirus system, it does not consider DIP formation and the passaging effect involved in continuous virus production in reactors [19]. While most of the model formulations focus on the dynamics of infected cells, uninfected and non-occluded virus formation [20, 25, 93], the packaging of nucleocapsids to form ODVs and the packaging of ODVs within polyhedra remain largely unaddressed.

Most of the experimental investigations done on baculovirus infection involve cell-disruptive approaches, such as gene expression using PCR and gel electrophoresis, Western blot, immunofluorescent assays for protein expression using immunofluorescent microscopy, studies on infected cell morphology, polyhedra production and measurement of viral size distribution using transmission electron microscopy. Live imaging of the infected cells and real-time monitoring of the dynamics of the intracellular processes using time-lapse imaging remain largely unemployed.

While the role of the various proteins regulating the modulation of the cell cycle and apoptosis has been investigated, to the best of our knowledge, specific models describing the modification of the cell cycle and apoptosis during baculovirus infection are not available. However, there are general models for apoptosis in virus infection and generic theories regarding the cell cycle. To fill this gap, an integrative approach needs to be taken to formulate a realistic model for the baculovirus infection process. Further, such models can be validated through fitting experimental data obtained at various scales, ranging from shaker flasks to bioreactors.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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